

***Ehrlichia canis* DNA in domestic cats parasitized by *Rhipicephalus sanguineus sensu lato* (s.l.) ticks in Brazil – case report**

Felinos domésticos parasitados por carrapato *Rhipicephalus sanguineus sensu lato* (s.l.) infectados por *Ehrlichia canis* no Brasil – relato de caso

Ísis Assis BRAGA^{1,2}; Isis Indaiara Gonçalves Granjeiro TAQUES¹; Jackeliny dos Santos COSTA¹; Ingrid Savino de Oliveira DIAS³; Estefânia Crivelatti GRONTOSKI³; Thaysa Felfili ZILIANI³; Andréia Lima Tomé MELO⁴; Daniel Moura de AGUIAR³

¹ Universidade Federal de Mato Grosso, Faculdade de Medicina Veterinária, Programa de Pós-Graduação em Ciências Veterinárias, Cuiabá – MT, Brazil

² Centro Universitário de Mineiros, Escola de Medicina Veterinária, Mineiros – GO, Brazil

³ Universidade Federal de Mato Grosso, Faculdade de Medicina Veterinária, Hospital Veterinário, Laboratório de Virologia e Rickettsioses, Cuiabá – MT, Brazil

⁴ Universidade de Cuiabá, Escola de Medicina Veterinária, Cuiabá – MT, Brazil

Abstract

Ectoparasites can transmit pathogens, including bacteria such as *Ehrlichia* sp., which trigger infectious diseases in domestic animals. Little is known about the epidemiology of feline ehrlichiosis, although several studies have focused on elucidating the pathogenesis and transmission of this disease. This paper presents the first mutual infection by *Ehrlichia* sp. between a domestic cat and a *Rhipicephalus sanguineus sensu lato* (s.l.) tick removed from the animal. The cat and tick were tested by Polymerase Chain Reaction (PCR) to detect the *dsb* gene, and the analyzed sequences revealed samples 100% identical to *E. canis*. Based on this report, we discussed the importance of cats as *E. canis* reservoirs and their position in the cycle of transmission between dogs and cats in Brazil.

Keywords: Cats. Ehrlichiosis. Tick-borne disease. Infection.

Resumo

Os ectoparasitos são capazes de transmitir patógenos incluindo algumas bactérias, como a *Ehrlichia* sp., causando doenças em animais domésticos. Pouco se conhece sobre a epidemiologia da erliquiose felina, embora alguns estudos já tenham sido realizados para elucidar a sua patogenia e transmissão. Este trabalho relata a primeira infecção mútua por *Ehrlichia* sp. entre um felino doméstico e o carrapato *Rhipicephalus sanguineus sensu lato* (s.l.) removido deste animal. Ambos foram testados pela Reação em Cadeia pela Polimerase (PCR) para detectar o gene *dsb*, e as sequências analisadas confirmaram amostras 100% idênticas à *E. canis*. Baseado neste relato é discutida a importância dos gatos como vetores de *E. canis* e sua posição no ciclo de transmissão de carrapatos entre cães e gatos no Brasil.

Palavras-chave: Gatos. Erliquiose. Doença transmitida por carrapato. Infecção.

Correspondence to:

Daniel Moura de Aguiar
Universidade Federal de Mato Grosso, Faculdade de Medicina Veterinária, Hospital Veterinário, Laboratório de Virologia e Rickettsioses
Av. Fernando Corrêa, 2367 – Boa Esperança
CEP 78060-900, Cuiabá, MT, Brazil
e-mail: danmoura@ufmt.br

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Rhipicephalus sanguineus ticks are important ectoparasites of domestic dogs and highly prevalent among canids throughout Brazil (LABRUNA; PEREIRA, 2001).

They are also competent vectors of *Ehrlichia canis*, which is responsible for Canine Monocytic Ehrlichiosis (CME) (MORAES-FILHO et al., 2015). Although *R. sanguineus* is highly prevalent in dogs in Brazil (LABRUNA; PEREIRA, 2001), some reports have also showed domestic cats parasitized by this tick species in the country (FERREIRA et al., 2009, 2010; MENDES-DE-ALMEIDA et al., 2011).

The presence of *E. canis* DNA has been reported in house cats and stray cats (BREITSCHWERDT et al., 2002; BRAGA et al., 2012, 2013; OLIVEIRA et al., 2009); however, natural cycles of transmission of this bacterium to cats have not been fully established (AMYX;

HUXSOLL, 1973). The present paper reports the discovery of parasitism of *R. sanguineus sensu lato* (s.l.) ticks on cats treated in a Veterinary Hospital in Brazil, and also the first simultaneous detection of DNA identical to *E. canis* in both the vertebrate and invertebrate hosts.

Eight domestic cats parasitized by ticks were treated at the Veterinary Hospital of the Federal University of Mato Grosso (HOVET-UFMT) between July 2013 and February 2014. Whole blood samples were collected for a complete hematological analysis and for the molecular detection of *Ehrlichia* sp. Ectoparasites were removed, analyzed under a stereoscopic microscope, identified according to the taxonomic key proposed by Barros-Battesti et al. (2006), and stored in polypropylene microtubes containing isopropanol. The Ethics Committee on Animal Use of UFMT, under Protocol No. 23108.019110/12-2, approved this study and its procedures.

The hematological analysis was performed according to the protocol specified by the Clinical Pathology Laboratory of HOVET-UFMT using the reference parameters of Jain (1993). For the molecular diagnosis genomic DNA was extracted from whole blood using AxyPrep Blood Genomic

DNA Miniprep Kits (Axygen BioScience, China), and genomic DNA was extracted from ticks using guanidinium thiocyanate, Sangioni et al. (2005). To amplify the fragment of *dsb* gene of *Ehrlichia* spp., a polymerase chain reaction (PCR) was performed according to Doyle et al. (2005). Amplicons were sequenced, generating sequences that were analyzed using the Basic Local Alignment Search Tool (BLAST) (ALTSCHUL et al., 1990) to check for identity with corresponding sequences available in the GenBank database.

Table 1 describes the age, sex, and breed of these cats, their clinical symptoms, parasitism by ticks, and detection of *Ehrlichia* sp. DNA by PCR. Ectoparasites were removed mostly from the head and neck, but also from ears, legs, and plantar area between the toes (Figure 1), and were identified as *R. sanguineus* s.l. ticks. Only one female of *R. sanguineus* s.l. had *Ehrlichia* sp. DNA. This tick was removed from an *Ehrlichia* sp. PCR-positive cat, which showed no clinical signs of infection (Table 1). Partial sequences of ehrlichial DNA generated from positive cats were analyzed and proved to be identical (100%) to each other and to *E. canis* sequences available in GenBank (GU586135.1, DQ460716.1).

Table 1 – Cats examined for clinical signs, parasitism by *Rhipicephalus sanguineus sensu lato* ticks, and detection of *Ehrlichia* sp. DNA by Polymerase Chain Reaction (PCR) – Cuiabá, MT, Brazil – 2017

Cat	Sex	Age (months)	Breed	Clinical exam/ hematological findings	Ehrlichial DNA from blood	Number of <i>R. sanguineus</i> / stage	Ehrlichial DNA from ticks
1	M	5	Mixed	No alteration	Present	1 M 1 F 1 N	Absent Present Absent
2	F	72	Persian	No alteration	Present	1 M	Absent
3	M	24	Mixed	Apathy	Absent	1 M	Absent
4	F	6	Persian	No alteration	Absent	8 L	Absent
5	F	2	Mixed	Apathy	Present	3 L 3 N	Absent Absent
6	M	3	Mixed	Dyspnea Paralysis of limbs	Absent	1 M	Absent
7	M	24	Mixed	Thrombocytopenia Dehydration	Absent	3 N 1 F 1 M	Absent Absent Absent
8	M	48	Mixed	Thrombocytopenia	Absent	1 F	Absent

F: female; M: male; N: nymph; L: larvae

Numerous authors have reported evidence of *E. canis* infection in domestic cats (BREITSCHWERDT et al., 2002; OLIVEIRA et al., 2009; BRAGA et al., 2012, 2013), which alerted veterinarians about the possibility of including feline ehrlichiosis as a differential diagnosis for cats with hematological disorders and parasitized by ticks. DNA

consistent with *E. canis* was detected in three cats infested with *R. sanguineus* s.l., although none of them presented altered clinical or lab values. The pathogenesis of *E. canis* in cats is not yet entirely clear, but in a previous study cats carrying ehrlichial DNA exhibited anemia, thrombocytopenia, lymphopenia, and monocytosis (BRAGA et al., 2013).

The description of parasitism by *R. sanguineus* s.l. in felines in the central-west region of Brazil coincides with reports of parasitism in cats (FERREIRA et al., 2009, 2010; MENDES-DE-ALMEIDA et al., 2011), which are rare since cats do not behave as primary hosts and usually remove attached ticks when grooming themselves (BREITSCHWERDT et al., 2002). The ticks removed from the cats in this study were located mostly on parts of the animals' bodies that were difficult to reach, making it hard to remove them. The tropical climate in the metropolitan region of Cuiabá is favorable for the maintenance of the vector *R. sanguineus* (RODRIGUEZ -VIVAS et al., 2005).

In addition to parasitism, the presence of *E. canis* DNA was detected in a tick removed from a PCR positive cat. The two sequences were identical to each other and to *E. canis* sequences, indicating they correspond to the same infectious agent. The transmission of *E. canis* by *R. sanguineus* ticks may be transstadial or intrastadial (BREMER et al., 2005). Based on these findings it was not

determined whether the presence of *E. canis* in ticks was a result of a blood meal on the infected cat, or if the infection was transmitted to the cat by a tick. This paper underscores the possibility that ticks act in the transmission cycle of *E. canis* to domestic cats, and is also the first report of *E. canis* DNA detected in a *R. sanguineus* s.l. tick parasitizing a mammal other than a domestic dog. Further studies are needed in order to evaluate the status of domestic cats in the epidemiology of *E. canis*.

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